# Other one-carbon micronutrients and age modulate the effects of folate on colorectal carcinogenesis

Joel B Mason, Sang-Woon Choi, and Zhenhua Liu



Epidemiologic observations as well as preclinical studies in animal and cell culture models indicate that diminished folate status increases the risk of carcinogenesis in certain tissues.[1,2] The biological plausibility of these observations is underscored by the fact that folate is an essential cofactor in biological methylation and nucleotide synthesis,[3,4] and it is presently believed that anomalies in DNA methylation and in DNA synthesis are among the most common molecular alternations that contribute to the development of human neoplasia.[5,6]

The data in this regard is most compelling for the colorectum, but increasing evidence is accruing for similar effects in the breast and possibly in other organs. However, as we go about fully defining this effect, it is important to remain cognizant of the fact that a variety of factors interact with folate status and thereby further modulate folate's effects on carcinogenesis.

## OTHER ONE-CARBON MICRONUTRIENTS AS CODETERMINANTS

The maintenance of normal patterns of biological methylation and nucleotide synthesis depends not only upon the adequate availability of folate but also on the adequate availability of other one-carbon nutrients, including vitamins $B_2$, $B_6$, and $B_{12}$.[7–9] These vitamins assume critical roles as cofactors in the one-carbon metabolic network in conjunction with folate. Thus, the metabolic functions of all these one-carbon vitamins are highly interdependent.

Aberrant signaling along the Wnt pathway is an early event in 90% of human colorectal cancers and is thought to play an important mechanistic role.[10] A variety of mutational and epigenetic silencing events in Wnt pathway genes, which ultimately effect a decrease in the degradation of β-catenin and increase catenin-mediated transcription of procarcinogenic genes, have been commonly identified in human colon neoplasia.[11,12] Classical mutations of *Apc* are often present, but *de novo* methylation of the *Apc* promoter region plays an important role as a second hit in silencing *Apc* expression in colorectal neoplasia.[13] We recently reported that *Apc* expression is also impaired by a severe degree of folate depletion.[4] Thus, evidence exists that Wnt signaling can be altered by dietary inadequacy of folate, albeit in response to a deficiency state of severe proportions.

Severe deficiencies of one-carbon nutrients are rare in the industrialized world. However, marginal status is common. This is particularly true for vitamins $B_6$ and $B_{12}$, and it appears that the elderly are among the most vulnerable.[14–16] We hypothesized that biochemical and molecular aberrations in the colon due to impaired one-carbon metabolism are magnified in the presence of mild inadequacies of several of these vitamins in a manner that would not otherwise be observed with folate depletion alone. We therefore designed a mouse study to examine the proposed synergies between these vitamins in regard to how they might impact on Wnt pathway. We intentionally used very mild levels of vitamin inadequacy in order to simulate the marginal inadequacies that are common in the general population. We also chose to study a mouse strain that is not predisposed to colorectal neoplasia, since we wished to examine the molecular and biochemical effects of these states of vitamin restriction in the absence of the confounding effects of a colon that is intrinsically driven towards neoplastic transformation.

Our results, which appear in detail in a recent publication,[17] confirmed our hypothesis. The induction of

Affiliation: *JB Mason*, *S-W Choi*, and *Z Liu* are with the Vitamins and Carcinogenesis Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, USA. JB Mason is also with the Divisions of Clinical Nutrition and Gastroenterology, Tufts-New England Medical Center, Boston, Massachusetts, USA.

Correspondence: *JB Mason*, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111, USA. E-mail: joel.mason@tufts.edu, Phone: +1-617-556-3194, Fax: +1-617-556-3234.

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DNA strand breaks in the colonic *Apc* gene, which developed in a specific manner since they were only observed to occur within the mutation cluster region of the gene, were associated with a significant fivefold reduction in *Apc* expression among those mice who were on the diet deficient in all four vitamins. An isolated depletion of folate or a depletion of folate and one other vitamin either did not produce a significant effect on *Apc* expression or tended to produce an effect of lesser magnitude than that observed with the multiple vitamin depletion state. No hypermethylation of the three CpG islands in the 5′ untranslated region of the gene was observed as a result of vitamin depletion, discounting this as a mechanism by which *Apc* expression could be suppressed.

Similarly, increased levels of celluar β-catenin in the colonic epithelium and increased translocation of the protein to the nucleus (which is a necessary event in canonical Wnt signaling), occurred to a greater degree in the multiple vitamin deplete state compared to singlet and doublet deficiencies. A pro-transformational gene, cyclin D1, whose expression is known to be upregulated by β-catenein-mediated transcription was increased more than threefold in the multiple deplete state but was not significantly increased by any of the other depletion states. Moreover, apoptosis, which is thought to be an important pathway by which the colonic epithelium avoids neoplastic transformation, was impaired to a significant degree by the multiple depletion state (35%) but not by any of the other singlet or doublet deficient states. In summary, perturbations in the Wnt-signaling cascade induced by the combined depletion of all four vitamins were consistently observed to be greater than those in the isolated folate depletion group, and in most instances were greater than the doublet states of depletion. This emphasizes the concept that diets which are inadequate in multiple one-carbon micronutrients may have important functional ramifications that do not exist with singlet or doublet states of depletion.

## ELDER AGE AS A CODETERMINANT

Another important factor that impacts folate metabolism is age. We have shown that the folate concentration within the colonic mucosa of elder rodents is only about one-half of the concentration observed in young adults fed equivalent amounts of dietary folate.[18] This differential only disappears when the animals are fed a diet containing four times the basal requirement of the vitamin. This effect of age appears to be tissue-specific since the differences between young and elder adults is not nearly as apparent when blood concentrations of folate are examined. Interestingly, the effect of age is not solely quantitative: the proportion of total colonic folate that is comprised of methylfolates is markedly diminished in the elder animals compared to the young adults. It is tempting to conclude that the susceptibility of the colon to folate depletion is related to the extremely high state of proliferation of this tissue. The elderly colon also appears to be more susceptible to changes in molecular aberrations that occur as a result of folate inadequacy: only the elder colon is susceptible to the induction of uracil incorporation as a function of folate depletion.[18] Similarly, genomic and p16 promoter methylation can be modulated by folate availability in the elder, but not young adult, colon.[19] Whether the effect of elder age as a major risk factor for colon cancer is in part conveyed by age-related changes in colonic folate metabolism is a matter of speculation but one that is ripe for investigation.

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